

What is claimed is:

1. A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing a cell which comprises a quorum sensing controlled gene, wherein said cell is responsive to a quorum sensing signal molecule such that a detectable signal is generated;

10 ~~contacting said cell with a quorum sensing signal molecule in the presence and~~
~~absence of a test compound;~~

and detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

15 2. The method of claim 1, wherein said cell further comprises means for generating said detectable signal.

3. The method of claim 2, wherein said signal generation means comprises
20 a reporter gene, and wherein said quorum sensing signal molecule causes transcription
of said reporter gene, said transcription providing said detectable signal.

4. The method of claim 3, wherein said reporter gene is operatively linked to a regulatory sequence of said quorum sensing controlled gene.

5. The method of claim 4, wherein said reporter gene is selected from the group consisting of *ADE1*, *ADE2*, *ADE3*, *ADE4*, *ADE5*, *ADE7*, *ADE8*, *ASP3*, *ARG1*, *ARG3*, *ARG4*, *ARG5*, *ARG6*, *ARG8*, *ARO2*, *ARO7*, *BARI*, *CAT*, *CHO1*, *CYS3*, *GAL1*, *GAL7*, *GAL10*, *GFP*, *HIS1*, *HIS3*, *HIS4*, *HIS5*, *HOM3*, *HOM6*, *ILV1*, *ILV2*, *ILV5*, *INO1*, *INO2*, *INO4*, *lacZ*, *LEU1*, *LEU2*, *LEU4*, *luciferase*, *LYS2*, *MAL*, *MEL*, *MET2*, *MET3*, *MET4*, *MET8*, *MET9*, *MET14*, *MET16*, *MET19*, *OLE1*, *PHO5*, *PRO1*, *PRO3*, *THR1*, *THR4*, *TRP1*, *TRP2*, *TRP3*, *TRP4*, *TRP5*, *URA1*, *URA2*, *URA3*, *URA4*, *URA5* and *URA10*.

35 6. The method of claim 5, wherein said reporter gene is *lacZ* or *GFP*.

7. The method of claim 1, wherein said cell does not express said quorum sensing signal molecule.

8. The method of claim 7, wherein said quorum sensing signal molecule is produced by a second cell.

9. ~~The method of claim 1, wherein said cell is a prokaryote or eukaryote.~~

10. The method of claim 9, wherein said cell is a bacterium.

11. The method of claim 8, wherein said second cell is a prokaryote or
10 eukaryote.

12. The method of claim 11, wherein said second cell is a bacterium.

13. The method of claim 10 or 12, wherein said bacterium is a gram negative bacterium.

14. The method of claim 13, wherein said gram negative bacterium is *Pseudomonas aeruginosa*.

15. The method of claim 10, wherein said bacterium is a mutant strain of
20 *Pseudomonas aeruginosa* which comprises a regulatory sequence of a quorum sensing
controlled gene operatively linked to a reporter gene, wherein in said mutant strain, *lasI*
and *rhlI* are inactivated.

16. The method of claim 12, wherein said second cell is wild type
25 *Pseudomonas aeruginosa*.

~~17. The method of claim 1, wherein said quorum sensing controlled gene is endogenous to said cell.~~

30 18. The method of claim10, wherein said quorum sensing controlled gene
encodes a virulence factor.

19. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which inhibits a bacterial host defense mechanism.

20. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which regulates biofilm formation.

Sub 32

5 lactone.

lactone analog.

10

Sub
A33

15

sensing signal molecule.

20

quorum sensing signal molecule;

25

30

compound as a modulator of quorum sensing signaling in *Pseudomonas aeruginosa*.

35

inactivated.

29. The method of claim 27, wherein said reporter gene is *lacZ* or *GFP*.
30. The method of claim 29, wherein said reporter gene is *lacZ*.
- 5 31. The method of claim 29, wherein said reporter gene is *GFP*.
32. The method of claim 31, wherein said reporter gene is a variant of *GFP*.
33. The method of claim 32, wherein said variant is GFPmut2.
- 10 34. The method of claim 27, wherein said mutant strain of *Pseudomonas aeruginosa* comprises a promoterless reporter gene inserted at a genetic locus in the chromosome of said *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID
- 15 NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID
- 20 NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36.
35. The method of claim 34, wherein said promoterless reporter gene is inserted in said chromosome at a locus comprising a nucleotide sequence selected from
- 25 the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:28 and SEQ ID NO:35.
36. The method of claim 34, wherein said reporter gene is contained in a transposable element.
- 30 37. A mutant strain of *Pseudomonas aeruginosa* comprising a promoterless reporter gene inserted at a genetic locus in the chromosome of said *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID
- 35 NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID

0953730:000100

NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36.

5

39.

10

41.

42.

43.

20

providing a wild type strain of *Pseudomonas aeruginosa* which produces a quorum sensing signal molecule;

25

30

35

contacting said mutant strain with said quorum sensing signal molecule and a test compound; and

- 5 detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in *Pseudomonas aeruginosa*.

45. The method of claim 44, wherein said reporter gene is contained in a transposable element.

10

46. An isolated nucleic acid molecule comprising a nucleotide sequence, said nucleotide sequence comprising:

15 a regulatory sequence derived from the genome of *Pseudomonas aeruginosa*, wherein said regulatory sequence regulates a quorum sensing controlled genetic locus of the *Pseudomonas aeruginosa* chromosome, and wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; and

25

a reporter gene operatively linked to said regulatory sequence.

47. An isolated nucleic acid molecule comprising a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa*, wherein
30 said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID

35

48. An isolated nucleic acid molecule comprising a polynucleotide having at least 80% identity to a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a reporter gene.

51. A vector comprising the isolated nucleic acid molecule of any one of claims 46, 47, 48 and 49.

53. A method for identifying a modulator of quorum sensing signaling in
5 bacteria, said method comprising:

10 contacting said cell with a quorum sensing signal molecule in the presence and
absence of a test compound;

15

55. The compound of claim 54, which inhibits quorum sensing signaling in *Pseudomonas aeruginosa* by inhibiting an enzyme involved in the synthesis of a quorum sensing signal molecule, by interfering with quorum sensing signal reception, or by scavenging the quorum sensing signal molecule.

providing a cell which is responsive to a quorum sensing signal molecule such that expression of a quorum sensing controlled gene is modulated, and wherein modulation of the expression of said quorum sensing controlled gene generates a detectable signal;

and detecting a change in the detectable signal to thereby identify a quorum
35 sensing signaling controlled gene in bacteria.

57. The method of claim 56, wherein said cell further comprises means for generating said detectable signal.

Variable	Mean	SD	Min	Max
Age	38.5	12.5	25	65
Gender	0.5	0.5	0	1
Marital Status	0.7	0.5	0	1
Education	12.5	2.5	9	16
Income	3500	1500	1000	8000
Health Status	0.8	0.4	0	1
Exercise Frequency	2.5	1.5	0	5
Stress Level	4.5	1.5	1	7
Sleep Quality	3.5	1.5	1	5
Diet Quality	3.0	1.5	1	5
Work-Life Balance	3.0	1.5	1	5
Life Satisfaction	4.0	1.5	1	7
Overall Well-being	4.5	1.5	1	7

58. The method of claim 57, wherein said signal generation means comprises a reporter gene, and wherein modulation of the expression of said quorum sensing controlled gene modulates transcription of said reporter gene, said transcription providing said detectable signal.

59. The method of claim 58, wherein said reporter gene is operatively linked to a regulatory sequence of said quorum sensing controlled gene.

60. The method of claim 58, wherein said reporter gene is opertively linked to said quorum sensing controlled gene.

61. The method of either of claims 59 and 60, wherein said reporter gene is contained in a transposable element.

62. The method of claim 58, wherein said reporter gene is selected from the group consisting of *ADE1*, *ADE2*, *ADE3*, *ADE4*, *ADE5*, *ADE7*, *ADE8*, *ASP3*, *ARG1*, *ARG3*, *ARG4*, *ARG5*, *ARG6*, *ARG8*, *ARO2*, *ARO7*, *BARI*, *CAT*, *CHO1*, *CYS3*, *GAL1*, *GAL7*, *GAL10*, *GFP*, *HIS1*, *HIS3*, *HIS4*, *HIS5*, *HOM3*, *HOM6*, *ILV1*, *ILV2*, *ILV5*, *INO1*, *INO2*, *INO4*, *lacZ*, *LEU1*, *LEU2*, *LEU4*, *luciferase*, *LYS2*, *MAL*, *MEL*, *MET2*, *MET3*, *MET4*, *MET8*, *MET9*, *MET14*, *MET16*, *MET19*, *OLE1*, *PHO5*, *PRO1*, *PRO3*, *THR1*, *THR4*, *TRP1*, *TRP2*, *TRP3*, *TRP4*, *TRP5*, *URA1*, *URA2*, *URA3*, *URA4*, *URA5* and *URA10*.

63. The method of claim 56, wherein said quorum sensing signal molecule is produced by a second cell.

64. The method of claim 63, wherein said second cell is a prokaryote or eukaryote.

65. The method of claim 64, wherein said second cell is a bacterium.

66. The method of claim 56, wherein said cell is a prokaryote or eukaryote.

67. The method of claim 66, wherein said cell is a bacterium.

68. The method of either of claims 65 and 67, wherein said bacterium is a gram negative bacterium.

74. The method of claim 56, wherein said quorum sensing signal molecule induces the expression of said quorum sensing controlled gene.

add
a341

[illegible]